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McBride, Sebastian D.; Perentos, Nicholas; Morton, A. Jennifer

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A mobile, high throughput semi-automated system for testing cognition in large non-primate animals models of Huntington's disease

Sebastian D. McBride, Nicolas Perentos and A. Jennifer Morton*

Department of Physiology, Development and Neuroscience, University of Cambridge, Downing Street, Cambridge, CB2 3DY, UK.

* Corresponding author: ajm41@cam.ac.uk; tel. 01223 334057; fax. 01223 333840

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Abstract

Background

For reasons of cost and ethical concerns, models of neurodegenerative disorders such as Huntington's disease (HD) are currently being developed in farm animals, as an alternative to non-human primates. Developing reliable methods of testing cognitive function is essential to determining the usefulness of such models. Nevertheless, cognitive testing of farm animal species presents a unique set of challenges. The primary aims of this study were to develop and validate a mobile operant system suitable for high throughput cognitive testing of sheep.

New Method

We designed a semi-automated testing system with the capability of presenting stimuli (visual, auditory) and reward at six spatial locations. Fourteen normal sheep were used to validate the system using a two choice visual discrimination task (2CVDT). Four stages of training devised to acclimatise animals to the system are also presented.

Results

All sheep progressed rapidly through the training stages, over eight sessions. All sheep learned the 2CVDT and performed at least one reversal stage. The mean number of trials the sheep took to reach criterion in the first acquisition learning was 13.9±1.5 and for the reversal learning was 19.1±1.8.

Comparison with Existing Method(s)

This is the first mobile semi-automated operant system developed for testing cognitive function in sheep.

Conclusions

We have designed and validated an automated operant behavioural testing system suitable for high throughput cognitive testing in sheep and other medium-sized quadrupeds, such as pigs and dogs. Sheep performance in the two-choice visual discrimination task was very similar to that reported for non-human primates and strongly supports the use of farm animals as pre-clinical models for the study of neurodegenerative diseases.

1. Introduction

Much has been learnt from rodent experimental models of neurodegenerative diseases such as Huntington disease (HD), but recent scrutiny has suggested that rodent models are unable to recapitulate fully the complexity of the clinical features found in the human condition (JPND Working Group, 2014). In particular, rodent models have been criticised for their inability to model the complex neuropathological changes that occur during disease progression, especially in relation to cognitive function and aging. Many of these issues are resolved by using non-human primate models, but there are major ethical concerns, as well as high costs associated with using primates as models of long-term neurodegeneration (Morton and Howland, 2013). In response to these challenges, new research has focused on developing alternative large animal models of neurodegenerative diseases such as HD.

Large animal models of HD are currently being developed in two species, pig and sheep (Baxa et al., 2013; Jacobsen et al., 2010). Both species are recognised as having advantages over rodents . In particular, their long lifespan (10-20 years) make them very suitable for modelling the late onset and slow progression of HD. In addition, the cortex of these animals are gyrencephalic (convoluted), and other sub-cortical structures such as the basal ganglia (the brain region that deteriorates first in HD), are anatomically much more similar to the structures found in human brain than are those of rodents.

Cognitive decline is one of the key symptoms in HD, thus, tests of cognition are critical for monitoring disease progression (JPND Working Group, 2014). Indeed, one of the recommendations of the recent 2014 report from JPND is that a greater development of reliable behavioural and cognitive tests is necessary for the longitudinal assessment of the efficacy of therapeutic agents. Cognitive testing in farm animals, however, creates a new set of challenges. Firstly, since animals are best tested *in situ* within their normal husbandry environment (Bayne and Wurbel, 2014), any testing system needs to be adaptable for use in the farm setting. Secondly, behavioural testing needs to accommodate the ethological priorities of the animal, because environments that do not support normal behaviours can affect the results of cognitive tests (Garner et al., 2006). Thirdly, because of the size and strength of farm animals, any testing system needs to be able to withstand a higher level of physical demand than would normally be expected from laboratory equipment used with small animals. Our primary objective, therefore, was to meet these challenges and design an operant testing system that is relevant and reliable for high throughput cognitive testing of farm animal species.

2. System design and fabrication

2. 1 Rationale

We had four main design goals in mind when we designed of the system. We wanted to

1. Create an operant system that is ethologically relevant to medium-sized quadrupeds;
2. make the system semi-automated;
3. make a system that is mobile, easy to transport and easy to assemble;
4. be able to present a flexible range of cognitive tests relevant to HD and other neurodegenerative diseases.

Our first challenge was to design a system that could be used for operant tasks that are ethologically relevant to sheep (Garner et al., 2006). Sheep are gregarious ruminants that spend large portions of the day and night as a flock engaged in ambulatory grazing (Lawrence and Woodgush, 1988; Lynch et al., 1992). Thus, we decided to design a system that required the animal to perform an ambulatory circuit that would constitute the appetitive phase of the goal-directed operant response. For the majority of cognitive tests used in pre-clinical behavioural tests, sensory stimuli are presented to the animal as an operant cue, as a way of eliciting choice and action selection. Historically, these stimuli have been visual, irrespective of the primary modality of sensory perception of the species in question (Garner et al., 2006). As it happens, visual stimuli are particularly relevant to sheep (Kendrick, 2008; Kendrick, 1998; Lange et al., 1995; Piggins and Phillips, 1996) and visually-based operant tests have previously been piloted successfully (Doyle et al., 2010; Morton and Avanzo, 2011). However, sheep are also attentive to olfactory and aural stimuli with successful testing of olfactory discrimination (Baldwin and Meese, 1977) and auditory discrimination (Taylor et al., 2010) tests. In light of this sensory evidence, it was decided that the operant design would use vision as the primary modality but that, with minor modification, the system should also be flexible enough to accommodate the presentation of other types of stimuli (e.g. auditory) in the future.

The second priority was to make the system semi-automated, in order to limit confounds associated with the operator. We thought this could be achieved by using an array of sensors to locate the animal at key points within the system, in particular to designate the starting position and also to sense the animal's location at critical points of choice relating to the cognitive task.

The third priority was to make a system that is mobile, easy to transport and easy to assemble in a farm setting. The key to meeting this objective was n the choice of materials, which had to be light enough to be moved by 1-2 people without additional equipment, but strong enough to withstand the repeated passage of animals that weigh up to 120kg in weight. In addition, we wanted it to be easily assembled by a small number of people (1-2). Furthermore, because of the size and strength

of the animals, the design needed to be constructed using robust fixtures that would not break under reasonable duress.

The fourth priority was to build a system that could be used to present a flexible range of cognitive tests relevant to HD but that could also be useful for testing cognitive function in other neurodegenerative disease models. Table 1 presents an analysis of cognitive tasks that are used to test HD patients (Cantab® HD cognition battery) as well as those used in rodent models of HD (Trueman et al., 2012a). We considered whether or not each test was currently being used for testing of non-human primates, and whether they might be useful for testing in sheep. As a result of this process, we decided that the design needed to allow different stimuli to be presented in multiple locations within the system with food reward also deliverable at those points. We also considered it important that the software running the cognitive tests should be adaptable in order to allow the full range of tests to be presented.

2. 2. Fabrication

The system was designed to have three expanded areas within a 8.7 x 3.1m arena (Figure 1). The first was a starting area where animals waited prior to beginning the cognitive test. The second was the ambulatory circuit area where the animal would engage and then disengage with stimuli. The third was the area where the stimuli and reward(s) were presented. The starting area had gates that allowed animals into the testing area. The ambulatory loop contained a central corridor to direct animals towards the stimuli and a transit area through which they would move at the end of each trial. The one-way direction of travel through this area was maintained using one-way gates (IAE, Stoke on Trent, UK). The central corridor contained a diffuse-reflective photo-electric sensor (Omron, Nufringen, Germany) that, when triggered, initiated the start of each trial (Figure 2a,b). Within the stimuli/reward area, 3 walls formed the back of the area (Figure 2a, b). This gave the

capacity for up to 6 regions to be created where both stimuli and reward could be presented. Visual stimuli were presented via liquid crystal display (LCD) screens (Dell, UK). The animal's choice was registered when it moved directly in front of a screen thereby triggering the infrared sensors situated above each screen (Figure 2a, b). The reward was delivered to a trough directly under the screens via a feed dispenser (Figure 3). Feed-dispensers were designed in-house and custom-built (Quality Equipment,Woolpit, UK) with a specification for 6mm sheep pellets with approximately 5-7g of pellets per dispense. The quantity of pellet delivered was determined to be a day ration (200g) divided by the maximum number of trials we predicted would be conducted in one day of testing (40). Feed-dispensers were designed so that the type and quantity of delivered reward could be varied. The dispensers have been used successfully by us to dispense pellets, dried peas, and barley. Feed-dispensers were designed to operate from a direct current power source (24v). The latter was specified in order to reduce the amount of electrical shielding required if the operant system was to be used in conjunction with electrophysiological experiments.

To make the system mobile, easy to transport and easy to assemble, the main structure of the system was fabricated using modular 1m high Paneltim plastic sheets (Paneltim, Lichtervelde, Belgium). This allowed the whole system to be flat packed in a single pallet-based container (3 x 1.3x 1.6m; 800kg) that could then be transported using standard haulage. The modular nature by which panels could be fitted together allowed one person to assemble the system within 8 hours.

Paradigm logic was processed using Matlab R2015a (Mathworks, UK) in conjunction with Psychtoolbox (Psyctoolbox.org) with inputs from sensors and outputs to dispensers relayed via a 12 bit USB data acquisition device (DAQ)(MCC 1208fs) (Measurement Computing, Norton, USA) (Figure 3). This arrangement of software and hardware gave flexibility for designing cognitive paradigms where several inputs (sensors) and outputs (screens, speakers, food dispensers) are required. In particular, the use of Matlab software provided a dynamic capability to alter the cognitive paradigm in response to the animal's behaviour during the course of any given trial. A general description of

the sequence of events during a generic trial are illustrated in Figure 4. In brief, the photo-electric starting sensor in the central corridor relays information about animal position to the DAQ device. This start signal is converted to a logic value that inputs to Matlab, which then commands the output of visual stimuli and auditory stimuli in relation to the cognitive test. The choice of the animal at the point of the screens is relayed, via photo-electric sensors, to the DAQ device. Matlab interprets this information in the context of the set cognitive paradigm and, if appropriate, elicits a food reward via a standard TTL pulse generated by the DAQ device. Figure 4 also shows the actions of the sheep and the human operator at each stage of the Matlab processes. This clearly demonstrates the semi-automated nature of the system where the human operator actions are limited to entry and exit of the animal.

3. Behavioural testing

By way of validating the system, 14 sheep were tested using a two-choice visual discrimination task that was modified from a protocol we had used previously to test cognitive function in sheep (Morton and Avanzo, 2011). Specifically, we wanted to confirm that the in-built ambulatory circuit was ethologically relevant for sheep, and secondly, that the automation and integration of sensors, screens and food dispensers worked to create a fluid cognitive test to produce optimal and efficient learning.

3.1 Animals

We used 14 mixed sex Borderdale sheep (9 females aged 37 ± 0.76 months, 5 castrated males aged 25±0.22 months). During the experiment, all animals were kept outdoors with free access to water, grazing and a field shelter. Sheep were given a feed supplement in the form of a standard ration of 200g cereal-based pelleted concentrate per day (Dodson and Horrell Ewe

and Lamb nuts, Dodson and Horrell, UK). On testing days, these pellets were provided as the food reward within the operant task. The female sheep had previously been used in a spatially-orientated operant study (McBride et al., 2014). Studies were carried out in accordance with the UK Animals (Scientific Procedures) Act, 1986. All animals came from and remained as permanent stock held at the University of Cambridge where the experimental work was carried out.

3.2 Acclimation and Training

In the acclimation phase, animals were fed pellets from buckets in the operant system, first as a single group (1 x 15 minute session), then as sub-groups of 7 (2x 15 minute sessions) and then groups of 3 (1 x15minute sessions). Finally, animals were fed as pairs within the system, with pellets dispensed from the feed-dispenser (1 x15minute sessions) by the operator.

Four stages of training to use the screens were developed, based on previous work training rodents within operant systems (Bussey et al., 2008b; Morton et al., 2006a). All animals were trained singly in each of the 4 stages. For all training stages, visual stimuli were presented using two LCD screen at screen positions 1 and 2 (Figure 2).

Stage 1 (2 sessions)

Purpose: To habituate and condition positively the animal to working in the operant system alone, and to expose it to the two points of reward delivery.

For each trial, two visual stimuli, randomly chosen from a library of 10 images modifed from the wingding font (Microsoft, U.S.A), were presented simultaneously with one stimulus on each screen. The visual stimuli presented were paired with simultaneous presentation of an audible tone (750Hz,

0.5s) and delivery of food from both dispensers every 10 seconds. Each session consisted of 10 presentations of stimuli with dispensing of the food reward. During Stage 1 training, the animal remained in the stimulus/reward area. No operant response was required to elicit a food reward. The end of the session was indicated by a prolonged low-pitched audible tone (260Hz, 1.9s). The total session time for each animal was approximately 4 minutes.

Stage 2 (2 sessions)

Purpose: To promote trial and error behaviour between the two points of reward delivery and to condition this behaviour to the presentation of visual stimuli on the screeens.

For each trial, one visual stimulus, randomly selected from a library of 10 wingding images was pseudo-randomly presented on one screen (left or right) with simultaneous presentation of an audible tone (750Hz, 0.5s). Animals were required to move to the screen carrying the image in order to trigger the sensor and elicit a food reward. There was no time-limit within which the animal needed to move to the correct screen. The inter-trial interval was 15 seconds with 10 trials in one session. During Stage 2 training, the animal remains in the stimulus/reward area. The end of the session was indicated with a prolonged low-pitched audible tone (260Hz, 1.9s). The total session time for each animal was between 3-6 minutes.

Stage 3 (3 sesssions)

Purpose: To introduce and acclimitise the animals to the one-way ambulatory circuit within each operant trial.

For each trial, one visual stimulus, randomly chosen from a library of 10 wingding images, was pseudo-randomly presented on one screen (left or right) with simultaneous presentation of an

audible tone (750Hz, 0.5s). Animals were required to move to the screen carrying the image in order to trigger the sensor and elicit a food reward. The animal was guided by a human operator out of the stimulus/reward area into the transit area via the non-return gate. The animal was then guided back to the stimulus/reward area area via the central corridor (Figure 1). One trial consisted of one loop through the ambulatory circuit with presentation of the stimulus and the food reward. Each trial was initiated by the shepp triggering the starting sensor within the central corridor. There were 10 trials in one session. There was no time-limit within which the animal needed to move to the correct screen nor was there any consequence of choosing the incorrect screen. The end of the session was indicated by a prolonged low-pitched audible tone (260Hz, 1.9s). The total session time for each animal was approximately 6-8 minutes.

Stage 4 (1 session)

Purpose: To intoduce the animals to the concept and consequence of error during the operant task.

For each trial, one visual stimulus, randomly chosen from a library of 10 wingding images, was pseudo-randomly randomly presented on one screen (left or right) with simultaneous presentation of an audible tone (750Hz, 0.5s). Animals were required to move to the screen carrying the image in order to elicit a food reward. Between trials, the animal was required to exit the stimulus/reward area into the ambulatory circuit area via the non-return gate and to then return to the stimulus/reward area via the central corridor. Trials were initiated when sheep triggered the starting sensor within the central corridor. This stage had 10 trials in one session. There was no time-limit on the animal moving to the correct screen. There was now, however, a consequence of choosing the incorrect screen. This led to the presentation of a high pitched audible tone (1000Hz, 0.5s), the image being removing and the animal being required to reinitiate the trial by moving out of stimulus/reward area, into the ambulatory circuit area and back through the central corridor. Since

259 animals within this stage of training could now make correct or incorrect reposness, the number of
260 correct trials (animals choosing the single stimulus) was recorded.

261 The end of the session was indicated with a prolonged low-pitched audible tone (260Hz, 1.9s). The
262 total session time for each animal was approximately 6-8 minutes.

263

264 *3.3 Two-choice visual discrimination task*

265 The two-choice visual discrimination task consists of the concurrent presentation of two visual
266 stimuli (A, B), one of which (S+) leads to the presentation of a reward. Both stimuli were presented
267 concurrently on two screens (pseudorandomly; 50% left, 50% right, position 1 and 2, Figure 2) with
268 simultaneous presentation of an audible tone (750Hz, 0.5s). For half the subjects (pseudorandomly
269 allocated), stimulus A was the S+ and for the other half B was the S+. A correct response elicted a
270 food reward and an incorrect response resulted in the presentation of a high pitched audible tone
271 (1000Hz, 0.5s) and no food reward. An incorrect response also resulted in the animal moving onto
272 'correction' trials (a repeat of the the incorrect trial) until a correct reponse was given. Correction
273 trials prevented strategies of side-bias where the animal would consistently choose one side in order
274 to attain 50% of the total reward (Horner et al., 2013). Each trial was time-limited to 45 seconds
275 after which a high pitched audible tone (2250Hz, 0.3s) was sounded and the trial ended. Each
276 session consisted of 10 trials (stimuli presentations). The end of the session was indicated by a
277 prolonged low-pitched audible tone (260Hz, 1.9s). Learning criterion was set at either 6 consecutive
278 (p=0.015) or 9 out of 10 (p=0.01) correct responses. Animals continued on the acquistion learning
279 phase until they had met criterion. Once animals had reached criterion for the first acquisition
280 (Acq1), the S+ and S- were reversed (Rev1). Animals continued on the reversal learning phase until
281 they met criterion. They were then tested upon a second set of novel stimuli (Acq2) and when they

had reached criterion they moved onto the second reveral (Rev2). This process continued for up to 3 acquistion phases during the course of 13 sessions with one session being carried out per day.

3.4 Statistics

All data are presented as mean ± sem. Significant differences were assessed using unpaired Student's t test or by one-way analysis of variance (ANOVA) with Newman Keuls post-hoc test where applicable. Statistical significance was set at $p \leq 0.05$.

4. Results

4.1 Acclimation and Training

All animals successfully completed the pre-training and training phases. The first two stages of training were set up to propagate trial and error type behaviour (moving between the two screens and food dispensers). Animals were observed to perform this behaviour primarily during Stage 2 when food was only dispensed once the animal triggered the sensor. Stage 3 training appeared to be the most difficult for some animals, with some animals becoming reactive to the presence of the human operator entering into the stimulus/reward area in order to move around the one-way system. This was resolved by having the operator maintain a passive body stance, avoiding sudden movement, maintaining a minimum distance from the animal (>2m) and always allowing the animal to keep the human operator within its field of vision. The mean number of correct responses during Stage 4 of training (7.93±0.58) was recorded as an indirect indicator of attentiveness to the visual stimulus.

4.2 Two-choice Visual Discrimination Task

All 14 animals completed the first acquisition phase (Acq1), reaching criterion within a mean of 13.9±1.5 trials. Most (13/14) animals also completed the first reversal phase (Rev1) taking a mean of 19.1±1.8 trials to reach criterion. For the second set of stimuli, 12/14 animals completed the second acquisition phase (Acq2) in a mean of 15.1±2.6 trials and 9 animals managed to complete the second reversal phase (Rev2) in a mean of 16.2±2.6 trials (Figure 5a). It is considered that all animals would have eventually completed both sets of stimuli if the task had not been time-limited to 13 sessions. For the 9 animals that completed to both pairs of stimuli, there was no significant difference in the number of trials to reach criterion between the two acquisition phases, nor between the two reversal phases. We also compared the number of correct choices in the last session of acquisition (when animals had reached criterion), and the first session of reversal for both set of stimuli (Acq1-Rev1 and Acq2-Rev2) (Figure 5b). As expected if learning had taken place, there was a significant drop in the number of correct responses from 89.2±1.8% to 25.4±4.2 for Acq1-Rev1 and from 89.1±2.1 to 25.0±4.0 for Acq2-Rev2 (Figure 6). Figure 6 presents example session-by-session data for 4 individual sheep and Figure 7 presents the mean session-by-session data for all animals. The data for the latter figure have been standardised over time, that is to say, once an animal has reached criterion within a phase, a value of 90% was assigned to that animal until all of the others reached criterion for that phase. Both figures clearly show the significant drop in the number of correct trials at the beginning of each reversal to below chance (as would be expected if learning had taken place), and a drop to the chance level at the start of acquisition phase for the second set of stimuli (as would be expected for a novel pair). An example of a sheep performing the two-choice visual discrimination task is presented in Video 1.

Of the 5 animals that did not complete the task using two sets of stimuli, two animals stopped responding after the first reversal phase. These animals were put into the arena each day and had

the opportunity to run the task for the duration of the 13 sessions but would not respond to the visual stimuli. Instead, after passing through the central corridor, they would stand in the stimulus/reward area and direct their attention towards the human observer with intermittent vocalisation until the trial timed-out. One animal continued to not respond to the stimuli for the duration of the 13 sessions. The other animal resumed performing after five sessions. After resuming, the latter animal then met the reversal criterion within 3 sessions. The other 3 animals did not complete two sets because they were slow.

5. Discussion

5.1 Mobile cognitive testing

The operant testing system was fully portable and quick and easy to assemble on site in a farm environment. The modular nature of the system meant that transport and assembly could be easily carried out by one operator. Testing and training was also easily achieved by one operator. Sheep readily adapted to the ambulatory circuit with all animals performing this automatically by the end of training stage 4. This meant that by the end of training there was very little need for action by the human operator. This achieved one of the four design goals. During Stage 4 of training, it was possible to record the number of correct trials where the animal went straight to the single visual stimulus presented on the screen. The mean performance level for all 14 animals during this stage was just below 80% suggesting that, after 7 training sessions (Stage 1-3), animals were already becoming highly attentive to the single visual stimulus within an operant context. In all, training was completed after 13 sessions (days) which is substantially shorter than has been reported for other species. For example, 47 daily sessions were needed to prepare marmosets for testing of an equivalent choice test (Adriani et al., 2013) and ‘several weeks’ of training for rhesus monkeys to perform a concurrent discrimination task (Voytko, 1999). The short duration of the training phase

suggested that the design of the operant system within this study was facilitating efficient learning. It also strongly supports the use of sheep as an easy and practical model for cognitive testing and neurodegenerative disease.

The use of Matlab code provided complete flexibility in terms of how, and when stimuli were presented, but it also allowed the paradigm to be changed at any point during the trial. This produced the desired aim of automation and thus minimised the opportunity of human operator influence on the animal's behaviour.

5.2 Two-choice visual discrimination task

As seen with the training data, the high percentage success rate for the first discrimination learning phases (93%) strongly suggested that the system design created a fluid cognitive test to produce optimal and efficient learning. This was supported by the speed at which animals reached criterion during the various stages of the test. On average fewer than two sessions of 10 trials were required for both the first acquisition and the first reversal (Figure 5a,b). This is significantly lower than that typically reported for rodents, where animals often take 9-15 sessions (30 trials) to reach criterion (Bussey et al., 2008a; Morton et al., 2006b). Notably, the performance level reported in this study was very similar to non-human primate studies. In a study by Rumbaugh (1971), gorillas, gibbons and talapoins reached criterion (9/10) for acquisition learning after an average of 1.6, 2.14 and 2.06 sessions of 10 trials respectively. This compares to 1.39 sessions for the sheep in this study. Similarly, after 8-11 sessions of reversal, gorillas had achieved 75% correct, gibbons 62% correct and talapoins 49% correct trials, whereas the sheep in this study required only 1.9 sessions to achieve to 90% correct trials. These data again provide strong evidence that large animal species such as sheep have a cognitive ability that makes them a viable alternative to non-human primates for the purposes of modelling cognitive dysfunction in neurodegenerative disorders.

We found the behaviour of the two animals that stopped responding after the first reversal phase to be particularly interesting. One of these animalas continued not to respond for the duration of the 13 sessions whilst the other animal resumed responding after five subsequent sessions. Both animals had performed well during the first acquisition phase with one animal requiring only one session to reach criterion and the other animal requiring four sessions. This suggests that the lack of response was due specifically to the reversal event. Both animals continued being exposed to the task, and although they would voluntarily enter the stimulus/reward area, rather than engage with the task, both would turn away from visual stimuli towards the human operator and intermittently vocalise. Although open to interpretation, these behaviours may suggest a negative emotional state that the animal links with the human operator. Interestingly, after five sessions, one of the animals started responding to the stimuli again and reached criterion for the reversal learning after three more sessions. This demonstrates that motivation to re-engage with the visual stimuli can be re-kindled after an animal has stopped responding. The presentation of a spontaneous reward (i.e. that not elicited by the actions of the animal) may be useful to reinstate operant responding in this respect. It may be advantageous, therefore, to include such an amendment into the operant code for future studies.

6. Conclusion

We have designed and validated an automated operant cognitive testing system suitable for high throughput testing of medium-sized quadrupeds. The system should be suitable for a range of cognitive tests relevant to HD or other neurodegenerative disorders and, because it is highly mobile, can be brought on-site to test animals in their home environment. The high success rate (whereby 93% of animals met criterion) and accelerated rate of learning (less than 2 sessions of 10 trials to reach criterion) during the two-choice visual discrimination task strongly suggested that the ambulatory circuit design of the system was ethologically relevant to sheep. It also demonstrated

that the automation and integration of sensors, screens and food dispensers worked to create a fluid system of cognitive testing that produced optimal and efficient learning.

Our mobile cognitive testing system has excellent potential for used for testing HD models (sheep and pigs). It also has substantial potential for research investigating cognition as a marker of the emotional state of farm and companion animal species (Burman et al., 2011; Douglas et al., 2012; Mueller et al., 2014; Pitteri et al., 2014). Finally, it could be used for studies of more general animal cognition such as those being undertaken in goats (Briefer et al., 2014; Langbein et al., 2007; Nawroth et al., 2015) and dogs (Mueller et al., 2014; Pitteri et al., 2014).

This study highlights the excellent potential for using sheep as an alternative large animal model to non-human primates, and strongly supports the use of sheep as models of neurodegenerative diseases in which cognitive function is impaired.

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Table 1. A critical comparison of cognitive tests currently used in the Huntington's disease battery for humans and rodents.

Task	Description	Used in non-human primate	Potential use in sheep
Human HD Task			
Two- choice visual discrimination task as part of Extra-intra-dimensional shift	Two-choice visual discrimination and reversal learning of visual object based on different rules e.g. shape and colour. Measures flexibility of learning and attention (Lawrence et al., 1998).	Yes (Dias et al., 1996)	Yes
Reaction time test	Motor response to the presentation of a visual cue in different spatial locations. Measures motor and mental response speeds (Jahanshahi et al., 1993).	Yes (Heimbauer et al., 2012)	No-lack of dextrous ability
One touch stockings of Cambridge	Visualisation of the number of actions to achieve a set goal. Involves spatial planning and working memory (Stout et al., 2014).	No	No-potentially too complex
Spatial Span	A number of empty boxes are presented on a screen and filled with colour in a particular sequence. Once the colour has been removed the subject must identify which boxes demonstrated a colour change (Lawrence et al., 1996).	Yes (Dudchenko et al., 2000)	Yes
Paired Associates Learning	Identification of location of different patterned objects that have been previously revealed and then occluded. Tests visual memory and learning (Rich et al., 1997).	Yes (Taffe et al., 2002)	Yes
Rodent HD Task			
Two- choice visual discrimination task	Two-choice visual discrimination and reversal of visual object based on different rules e.g. shape and colour. Measures flexibility of learning and attention (Morton et al., 2006a).	Yes (Dias et al., 1996)	Yes
5 choice serial reaction time test	Operant movement towards one of five briefly (e.g. 0.5s) lighted areas with errors of movement recorded during the inter-trial interval (e.g. 5s). Measures attention and impulsivity (Trueman et al., 2012b).	Yes (Weed et al., 1999)	Yes
Serial implicit learning task	Similar to the 5 choice serial reaction time test but subjects must respond correctly to two consecutive light stimuli. Tests implicit learning (Trueman et al., 2007).	Yes (Locurto et al., 2010)	Yes
Choice reaction time	Subjects wait in a learned location and then respond left or right to a visual	Yes (Emadi and Esteky, 2009)	Yes

task	cue (Cao et al., 2006).		
Delayed alternation	Spatially alternating operant response with delay between responses. Involves rule learning and memory (Trueman et al., 2009).	Yes (Levy et al., 1997)	Yes
Progressive ratio	The number of correct operant responses for a reward is increased progressively. The point at which the animal stops responding is referred to as the break point. Measures motivation and apathy (Trueman et al., 2009).	Yes (Roberts et al., 1989)	Yes
Peak Procedure	Subjects are trained to continuously respond for a delayed reward (e.g. 20 s). This results in a U shaped curve of responding with the peak at time of the learned reward presentation. This is a test to temporal processing (Balci et al., 2009).	Yes (Fiorillo et al., 2008)	Yes

Figure and Video Descriptions

Figure 1. A three-dimensional diagram of the mobile operant system. Blue arrows indicate the potential routes that can be taken by each animal during each trial.

Figure 2. a) Diagram of the front aspect of the three panels in the stimulus-reward area of the operant system. b) Photograph of an animal proceeding through the middle corridor towards the visual stimuli. The position of the start sensor within the corridor is indicated by the arrow.

Figure 3. Diagram of the operant system from the back. The monitoring of sensors and presentation of food via the dispensers is controlled by Matlab via the data acquisition (DAQ) device. The presentation of visual stimuli is controlled directly by the Psychtoolbox module within Matlab.

Figure 4. A diagram illustrating the flow of events during a generic cognitive test , showing the relationship between the animal, the logic of the Matlab code and the human operator.

Figure 5. A summary of two-choice visual discrimination task data for all sheep. a) Mean number of trials to criterion for each of the acquisition-reversal phase with two sets of stimuli. b) Mean percentage of correct trials during the last session of acquisition and first session of reversal for each set of stimuli.

Figure 6. Individual performances in the two-choice-discrimination task data of 4 sheep.

Figure 7. A session-by-session summary of the performance of all sheep. Data are the mean number (± s.e.m) of correct trials. Once an animal reached criterion, it was assigned a score of 90% until all remaining animals reached criterion within that acquisition or reversal phase.

Video 1. A Borderdale sheep performing the two-choice-visual discriminating learning task. The animal triggers the starting sensor within the central corridor and then proceeds to the two screens within the stimulus/reward area. Upon making the correct choice, a food reward is dispensed. The animal then completes the trial by exiting into the transit area whilst passing the human operator. The next trial begins

once the ambulatory circuit has been completed and the starting sensor in the central corridor is again triggered.

Fig 1 (double column, 190mm)

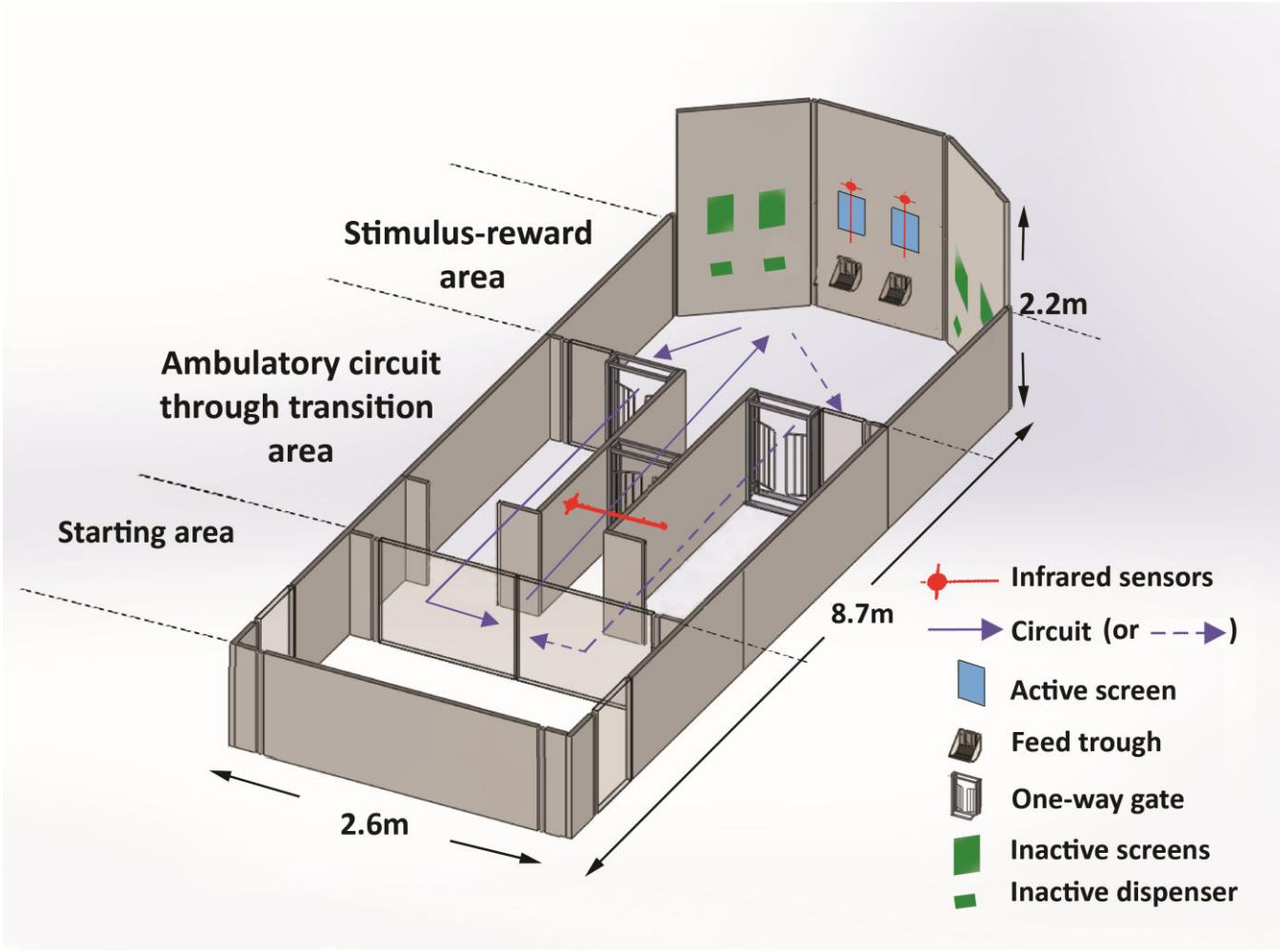
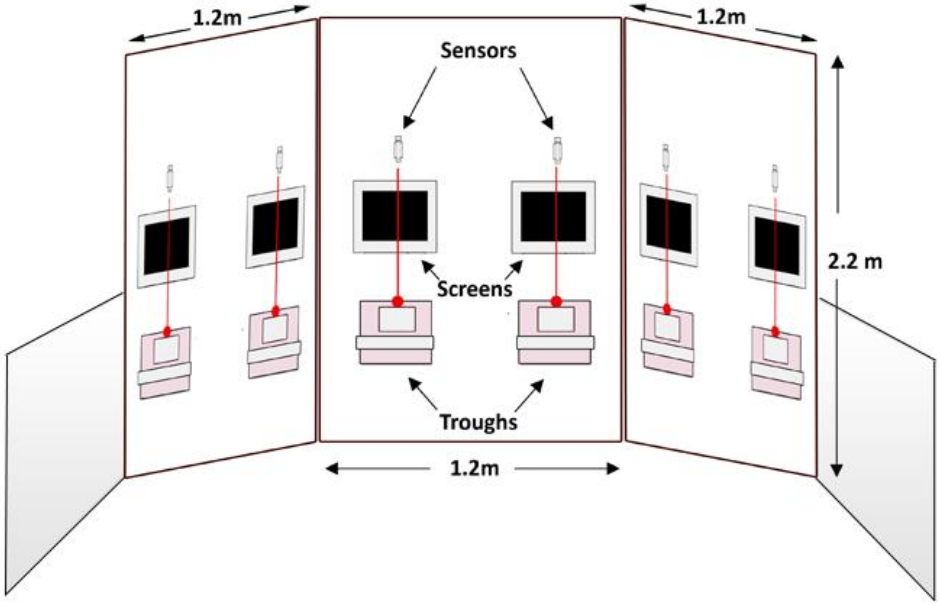


Fig 2 (1.5 column, 140mm)

a



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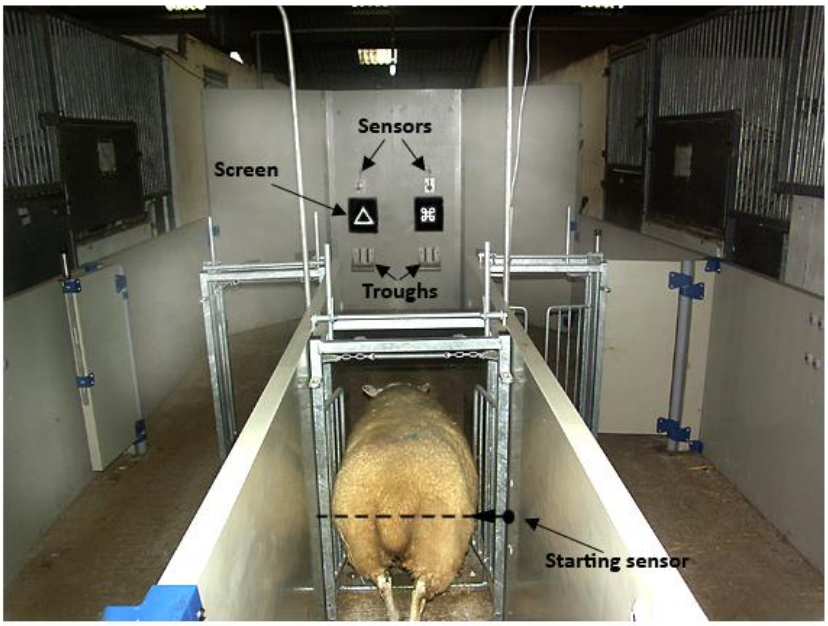

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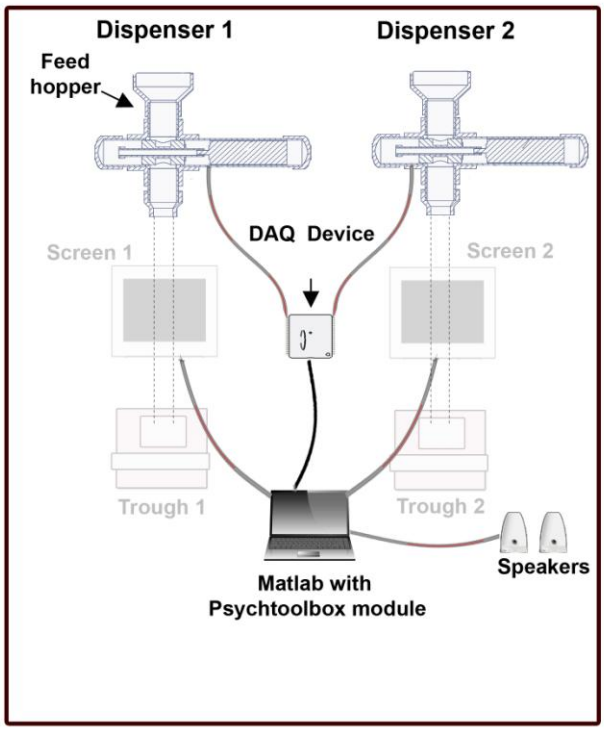

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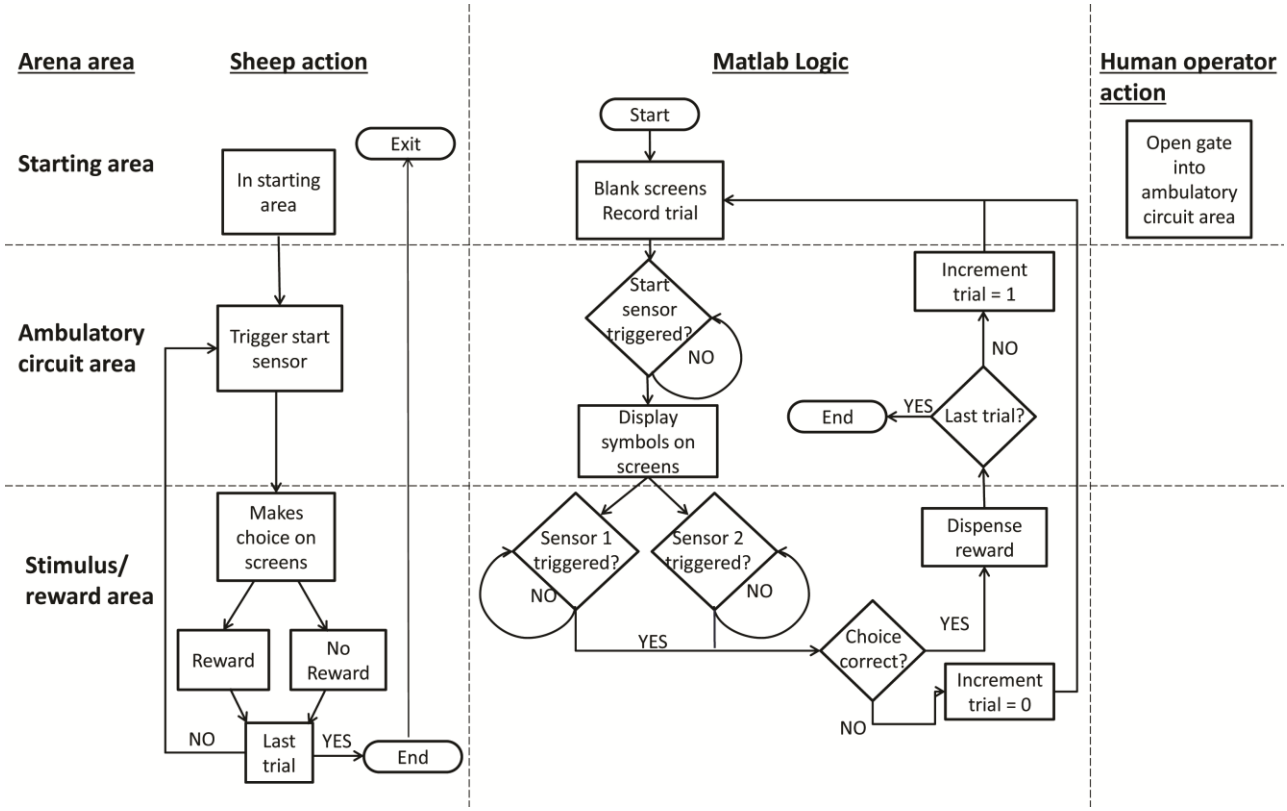

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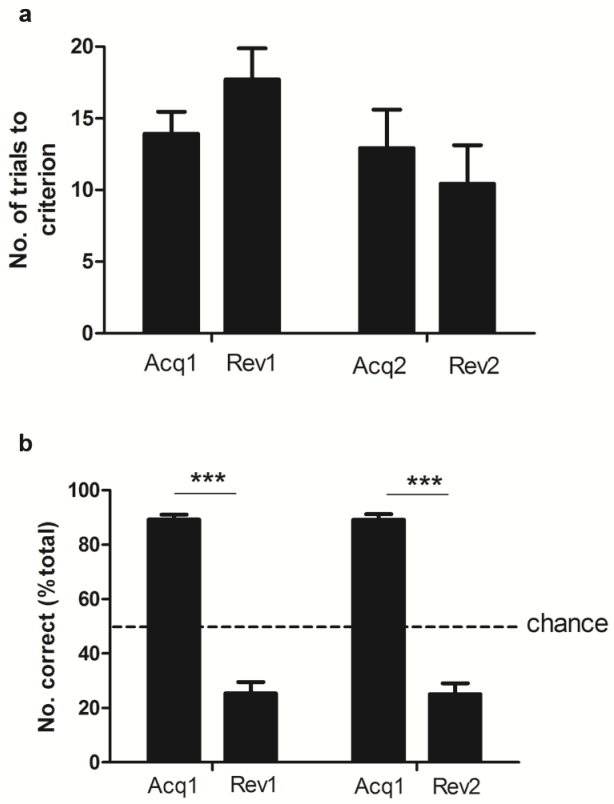

Figure 6 (1.5 column, 140mm)

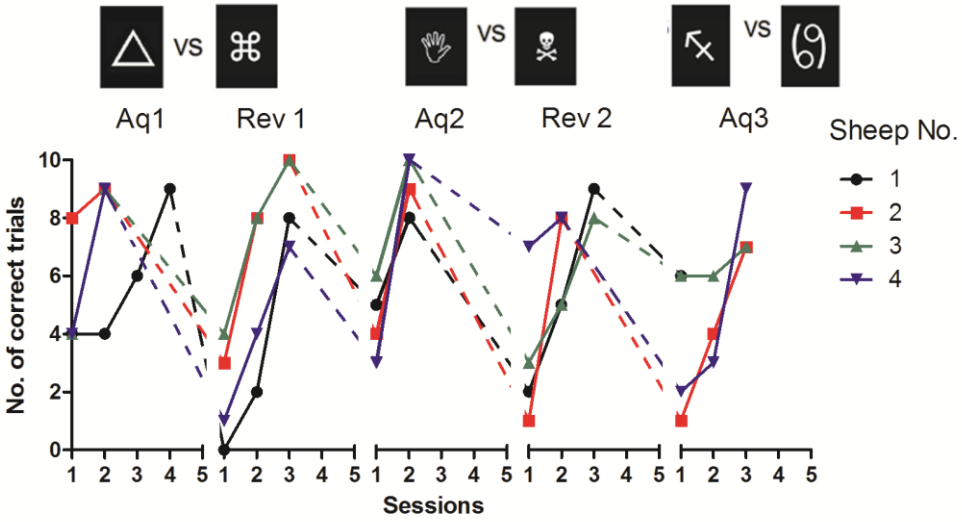

Figure 7 (1.5 columns, 140mm)